IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Jean-Marc Balloul et al.	Group Art Unit: 1648
Application No.: 09/506,942	Examiner: Shanon A. Foley
Filed: February 18, 2000	Confirmation No.: 9626
For: PHARMACEUTICAL COMPOSITION FOR TREATING PAPILLOMAVIRUS TUMORS AND INFECTION	

DECLARATION BY JEAN-MARC BALLOUL UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jean-Marc Balloul, hereby state as follows:

1. I am a named inventor on U.S. Application No. 09/506,942 ("the '942 application").

2. I believe and allege the '942 application is a divisional application of U.S. Application Serial No. 09/043,933 ("the '933 application"), filed March 30, 1998, which claims priority benefit of French Application No. 96-09584, filed July 30, 1996.

3. I believe and allege we conceived the invention claimed in the '942 application prior to March 1996, and that conception was pursued with diligence from the time it was conceived before March 1996 until we filed the priority application, French Application No. 96-09584, on July 30, 1996.

4. Prior to March 1996, I participated in preparing an internal report for a scientific council at Transgene, attached as Exhibit A. This report discusses the production of clinical batches of vaccina virus construct VVTG5021 & 5065. This report further demonstrates expression of HPV genes and IL-2, the absence of

toxicity seen in animal models following injection. The report also provides the same immunoprotein and immunotherapy assays as seen in Example 6 of the '942 application. The exact date of the internal report has been redacted on the copy provided. Further, data not connected to the '942 application has also been redacted on the copy provided.

5. Exhibit A, and the work detailed therein, was prepared in France, a World Trade Organization (WTO) member country, prior to March 1996.

6. We believe and allege each element of the claims of the '942 application is disclosed in Exhibit A.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: April 6th 2006

JEAN-MARC BALLOUL

EXHIBIT A

Project N°87
Research and Development
Scientific Direction: MPK
Project Leader: JMB
Molecular Biology and Virology: MG, KD, ReB, JMB
Pre-clinical studies: NaB, CHP, LS, GlG, LR, BA
Production and controls: KD, EB, OF, EK, DV, HeL, JMB

Cervical cancer associated with Human Papillomavirus

Among the 75 types of human papillomavirus types so far identified 20 dinstinct isolates are known to infect the genital tract and 4 types (HPV16, 18, 31 and 45) account for 80% of HPV infection associated with cervical cancer. Although cervical cancer rates have been declining, they remain relatively high in developping country where prevention is lacking. Both therapeutic vaccination (induction of the regression of precancerous and cancerous lesions associated with oncogenic HPV types), and prophylactic vaccination are under investigation in various laboratories throughout the world. Prophylaxis with HPV vaccines is certainly the most promising approach and will have a long term impact in reducing cervical cancer. At the moment a consensus exist for the choice of the immunogens to be include in prophylactic vaccines. They are in fact the virus-like particles (VLP) synthesised *in vitro*. These DNA free particles shown their ability to induce neutralizing antibodies. Several compagnies are now proceeding to the development and production of VLPs at the standards required for human trials.

Clinical batch for HPV16 invasive carcinoma immunotherapy

As we predict that successful vaccination against the human papillomavirus requires induction of both cytotoxic T lymphocyte (CTL) response against early viral proteins and neutralizing antibodies against capside proteins L1/L2 to prevent reinfection we developped a vaccinia virus expressing both early and late HPV antigens under the control of early late vaccinia virus promoters. Briefly HPV16 E7 and E6 have been mutated according the litterature in order to abrogate their transforming activity. Then mutant genes were inserted by homologuous recombination at the K1L locus of the vaccinia virus genome (Copenhagen strain) and placed under the control of the early late vaccinia virus promoter H5R. At the same locus we inserted both the human Il2 gene placed under the control of the early late vaccinia virus promoter p7.5 and a selection marker gene LacZ placed under the control of the K1L promoter (see figure 1). Genes encoding for the late viral proteins L1 and L2 were inserted into the TK locus of the vaccinia virus genome and placed under the control of the vaccinia virus early-late promoter p7.5 (see figure 2).

Clinical batch was produced in our L3 unit on primary CEF. The viral amplification leads to the production of more than 3,000 doses of vaccines containing around 10E8 pfu per doses. Expression of HPV genes was analyzed by Western blot analysis. As shown in figure 3, 4,5 6, HPV antigens are expressed and detectable by Western blot. Level of Il2 was measured and evaluated to 10ng/ml/24h/10E6 cells infected with 1pfu per cell (see figure 7). Complete sequencing of open reading frames showed in the case of Il2 the accidental cloning of a 600bp

DNA fragment corresponding to the β-globin intron present in the parental plasmid. As 4 ORFs encoding for small polypeptides (26 to 56 aa) could be taken in account by the early-late vaccinia virus promoter controllin the expression of the human Il2 gene we analyzed the presence of such contaminants in VVTG5021&5065 infected BHK-21 extracts. Results showed that no contaminants are detectable compared with VVTG5021&5061 extracts expressing all genes present in the VVTG5021&5065 except the human Il2 gene (see figures 8, 9).

New formulation of HPV16 early antigens in vaccinia virus

MUC1/B7.1 formulation

In order to enhanced immunigenicity of the MUC-1 antigen we constructed a new replicative vaccinia virus co-expressing the human B7.1 gene and the human MUC-1 cDNA(name code pTG6005 figure 13). MUC-1 cDNA available at Transgene contains more than 24 tandem repeats and regarding the propensity of the vaccinia virus genome to eliminate DNA repetitions we analyzed number of plaques after the first TK- selection. We isolate one clone containing at least 12 repeat units which is an impressive number compared to the VVTG5058 viral isolate.

Non Replicative Vaccinia Virus as Vector for Cancer Immunotherapy

Introduction

Vaccinia virus is the prototype of Orthopoxvirus. It can replicate in a wide range of cells. At least three viral genes affect the host range of orthopoxviruses in tissue culture: K1L gene, C7L gene and the cowpox 77-kDa gene. As regulatory authorities showed some fair concerning the use of replicative vectors for human health, we decided to develop attenuated poxvirus expression vectors. First question was to select the appropriate strain of vaccinia virus. Paolletti et al. has previously and extensively described various pox virus in their non replicative form such has NYCAC (highly attenuated vaccinia virus engineered from the Copenhagen strain) and ALVAC (Avian canaripox which is not replicative into mamalian cells). Unfortunatly vectors for homologous recombination into these non replicative poxvirus were not accessible for Transgene. In the same time G. Sutter in B. Moss laboratory (Laboratory of viral diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, USA) has described a recombinant vector derived from the host range-restricted and higly attenuated MVA strain of vaccinia virus which has been shown to express the inserted foreign gene in authentic fashion and to provide the antigen to the immune system in such maner that it induces a protective immunity against pathogen such like the influenza virus in mice when it expresses haemagglutinin and nucleoprotein genes.

MVA known as modified vaccinia virus Ankara was found to be avirulent in normal or immunosuppresses animals ranging from rodents to macaques and was without significant side-effects in 120,000 humans, many of whom were at high risk for conventional smallpox vaccine. During over 570 passages in chicken embryo fibroblasts, MVA became host-restricted and unable to grow in almost all tested mamalian cell lines including those of rodent and human origin. Analysis of the genome revealed that viral DNA had suffered six major deletions resulting in the loss of 30000 base pairs equivalent of 15% of its genetic information. The replication of MVA DNA implied that the initial stages of infection comprising viral attachment, entry, early gene expression, and uncoating occurred in non permissive human cells. G. Sutter et al. has oberved that only immature virus particles appearing as circular spiculecoated membranes encircling granular material and lacking dense nucleoprotein bodies could be seen by electron microscopic examination of human cells infected with MVA whereas mature brick-shaped particles with complex internal structures were numerous in human cells with WT or CEF with MVA. Moreover no increase in MVA titer was detected in Hela or 293 cell lines, and

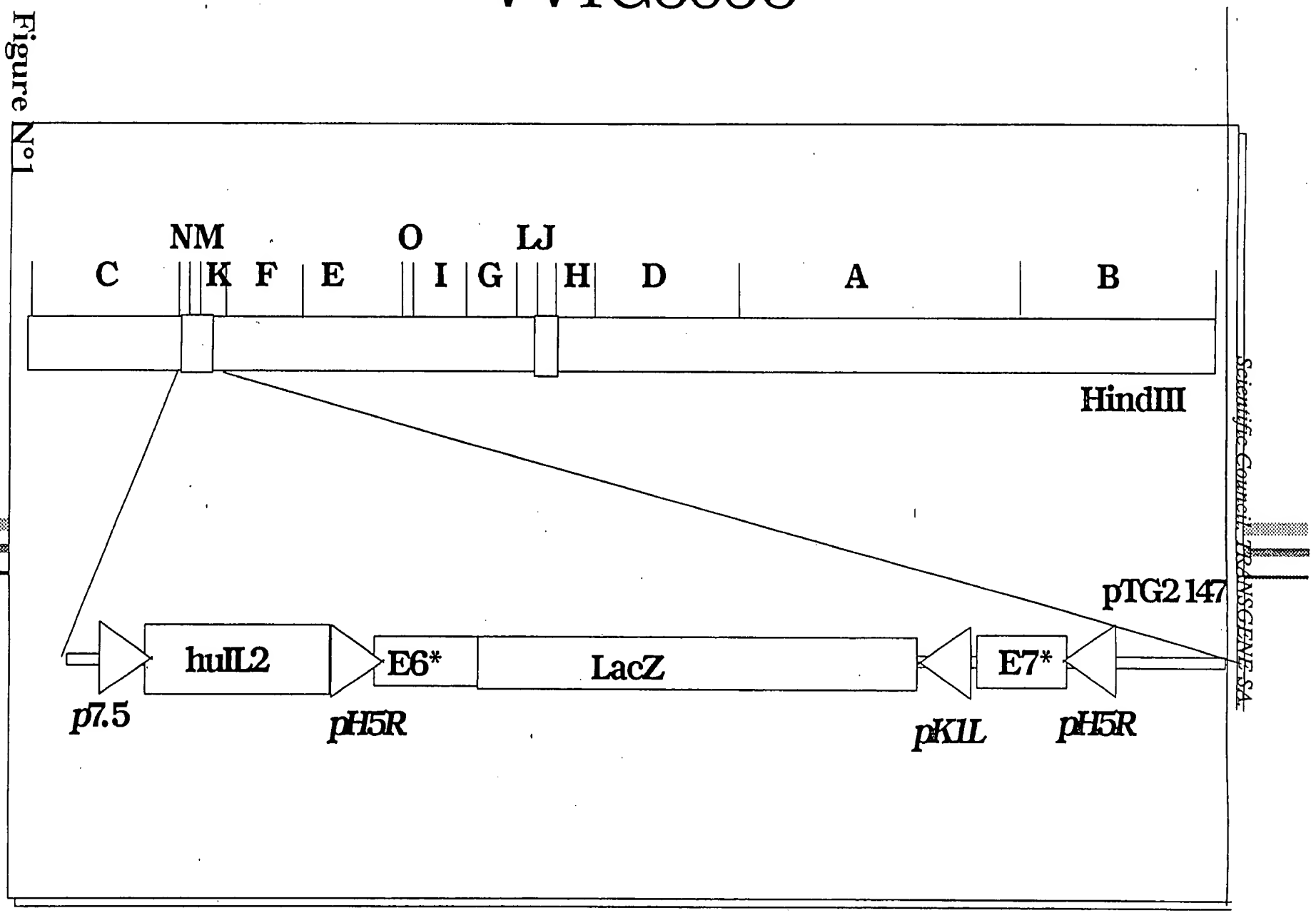
at the opposite WT increase 10,000-fold in titer in the same conditions. These differences are not associated with DNA replication because concatemeric forms of replicative MVA DNA are processes normally to unit genome. These authors observed common viral protein pattern for both WT and MVA in infected human cells with one exception for a 90kDa polypeptide wich is not seen in the MVA protein pattern. This is attributed to the deletion of the gene encoding nonessential A-type inclusion protein homolog. In pulse-chase assays they also showed that if in WT infected Hela cells expected cleavage of at least 5 polypeptides including the major core protein precursors P4a and P4b occured, the processing of MVA proteins was inhibited.

Plasmid construction

PCR segments flanking either deletion III or deletion II were cloned into pTG1E at the unique EcoRI site (code name pTG6018 containing flanking DNA segments for homologous recombination at the deletion II site and pTG6019 containing flanking DNA segments for homologous recombination at the deletion III site figure 14). In order to facilitate the isolation of recombinant viruses we inserted between these flanks a double expression cassette composed of the early late promoter p7.5 controlling the marker gene and of the early late promoter p7.5 in the same orientation controlling a reporter gene for internal recombination which encode for the human-Il2. We have selected as markers either the LacZ gene encoding β-galactosidase (β-gal) in the case of deletion II and the E. coli *gusA* gene encoding β-glucuronidase (GUS) in the case of the deletion III. β-gal and GUS being from E. coli origin we decided to eliminate these genes from the final clinical product. For these purpose we used the natural propensity of the vaccinia virus to eliminate DNA repeated sequences. Therefore p7.5 flanking marker gene should recombinate properly, excising the E. Coli gene and reconstituting a functional p7.5 promoter controlling the human Il-2 gene (figure 15).

VVTG5065

Figure N°1



Transgène S.A., 11 rue de Molsheim 67082 STRASBOURG, FRANCE

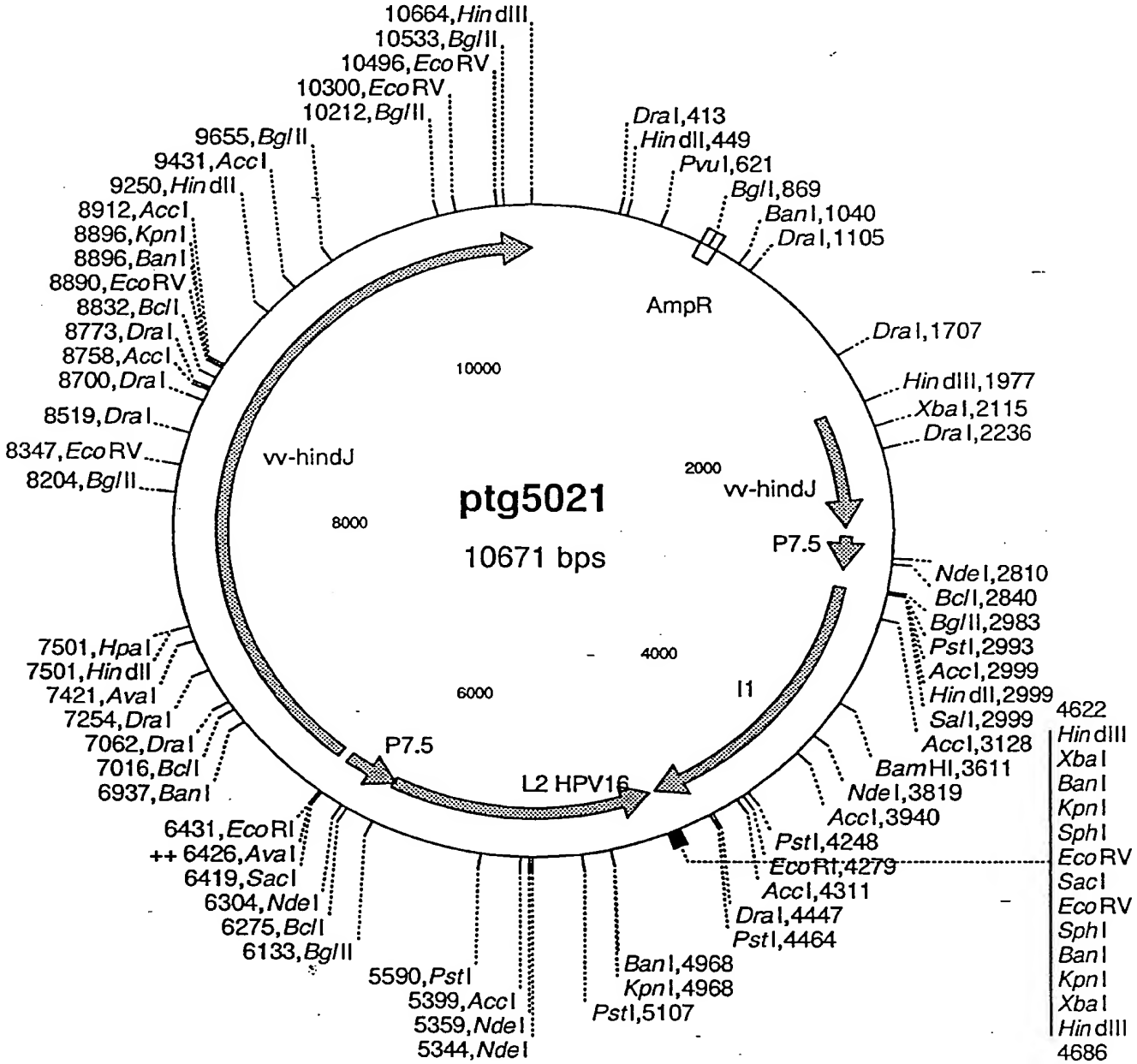
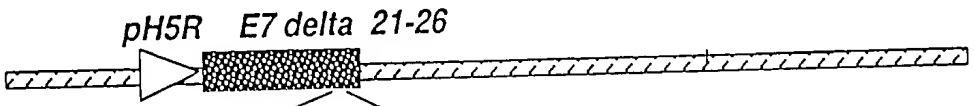

ptg5021
10671 bps
AmpR
vv-hindJ
vv-hindJ
P7.5
P7.5
L2 HPV16
I1
10000
2000
4000
6000
8000
10664,HindIII
10533,BglII
10496,EcoRV
10300,EcoRV
10212,BglII
9655,BglII
9431,AccI
9250,HindII
8912,AccI
8896,KpnI
8896,BanI
8890,EcoRV
8832,BclI
8773,DraI
8758,AccI
8700,DraI
8519,DraI
8347,EcoRV
8204,BglII
7501,HpaI
7501,HindII
7421,AvaI
7254,DraI
7062,DraI
7016,BclI
6937,BanI
6431,EcoRI
++ 6426,AvaI
6419,SacI
6304,NdeI
6275,BclI
6133,BglII
5590,PstI
5399,AccI
5359,NdeI
5344,NdeI
PstI,5107
BanI,4968
KpnI,4968
PstI,4464
DraI,4447
AccI,4311
EcoRI,4279
PstI,4248
AccI,3940
NdeI,3819
BamHI,3611
AccI,3128
SalI,2999
HindII,2999
AccI,2999
PstI,2993
BglII,2983
BclI,2840
NdeI,2810
DraI,2236
XbaI,2115
HindIII,1977
DraI,1707
DraI,1105
BanI,1040
BglI,869
PvuI,621
HindII,449
DraI,413
4622
HindIII
XbaI
BanI
KpnI
SphI
EcoRV
SacI
EcoRV
SphI
BanI
KpnI
XbaI
HindIII
4686

Figure N°2

Figure N°4

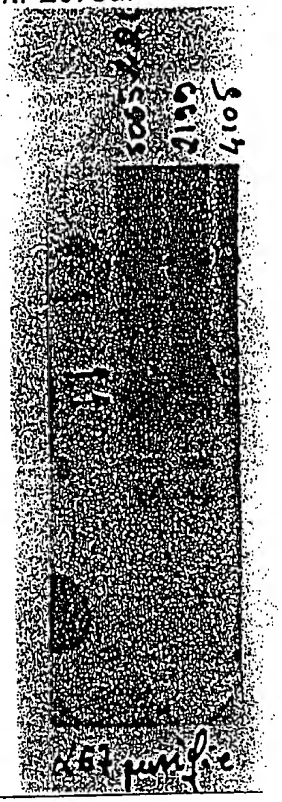
E7 delta 21-26 produite par VVTG5021&5065



Séquence pTG5021&5065

```
DNA Align (ALIGN) version 5.90 Apr 93
This is Align ~5065.SEQ(1,6829) with E7.SEQ(1,297) Ktuple: 3 Gap penalty: 3 Largest: 20

Alignment of:
C:\JMB\CANUTER\PL5065\~5065.SEQ     6829 bases from 1 to 6829
          versus
C:\JMB\CANUTER\PL5065\E7.SEQ     297 bases from 1 to 297

K-tuple size = 3     range = 20     Gap penalty = 3

            110v      120v      130v      140v      150v      160v      170v      180v      190v      200v
~5065    GGGTTGTGTTAAATTGAAAGCGAGAAATAATCATAAATTATTTCATTATCGCGATATCCGTTAAGTTTGGATCTGCAGTAATCATGCATGGAGATACACC
                                                                                                ATGCATGGAGATACACC
                                                                                                ATGCATGGAGATACACC
E7                                                                                                        10^

            210v      220v      230v      240v                                  250v      260v      270v      280v
~5065    TACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACT----------------CAATTAAATGACAGCTCAGAGGAGGAGGATGAAATAGAT
         TACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACT                CAATTAAATGACAGCTCAGAGGAGGAGGATGAAATAGAT
E7       TACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACTGATCTCTACTGTTATGAGCAATTAAATGACAGCTCAGAGGAGGAGGATGAAATAGAT
         20^       30^       40^       50^       60^       70^       80^       90^       100^      110^

            290v      300v      310v      320v      330v      340v      350v      360v      370v      380v
~5065    GGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACAC
         GGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACAC
E7       GGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACAC
            ^      130^      140^      150^      160^      170^      180^      190^      200^      210^

            390v      400v      410v      420v      430v      440v      450v      460v      470v      480v
~5065    ACGTAGACATTCGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAA
         ACGTAGACATTCGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAA
E7       ACGTAGACATTCGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAA
            ^      230^      240^      250^      260^      270^      280^      290^

Similarity index = 99.643%, total bases not opposite a gap = 279
Number of gaps = 1,  bases opposite gaps = 18
Length of the overlapping region = 297
Number of unambiguous matching bases = 279
Number of ambiguous matching bases = 0
```

WB anti-E67sur VVTG5021&5065

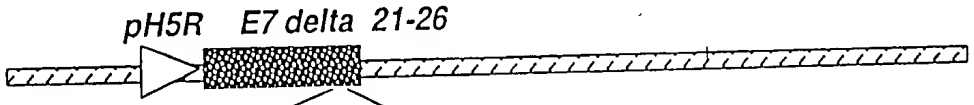


Scientific Council TRANSGENE SA

Eval10.95/87/131

Figure N°4

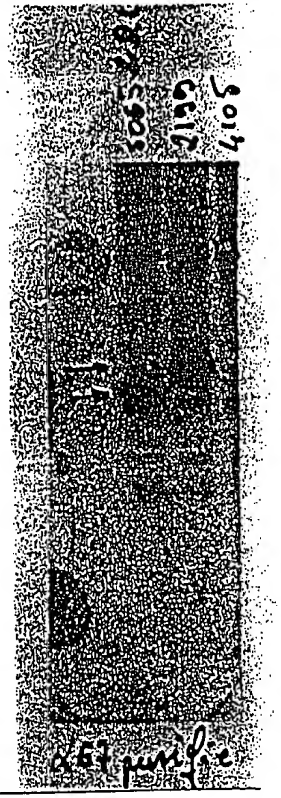
E7 delta 21-26 produite par VVTG5021&5065



Séquence pTG5021&5065

```
 DNA Align (ALIGN) version 5.90 Apr 93
This is Align ^5065.SEQ(1,6829) with E7.SEQ(1,297) Ktuple: 3 Gap penalty: 3 Largest: 20

Alignment of:
C:\JMB\CANUTER\PL5065\^5065.SEQ     6829 bases from 1 to 6829
          versus
C:\JMB\CANUTER\PL5065\E7.SEQ     297 bases from 1 to 297

K-tuple size = 3     range = 20     Gap penalty = 3

            110v      120v      130v      140v      150v      160v      170v      180v      190v      200v
^5065   GGGTTGTGTTAAATTGAAAGCGAGAAATAATCATAAATTATTTCATTATCGCGATATCCGTTAAGTTTGGATCTGCAGTAATCATGCATGGAGATACACC
                                                                                                ATGCATGGAGATACACC
                                                                                                ATGCATGGAGATACACC
E7                                                                                                        10^

            210v      220v      230v      240v                                  250v      260v      270v      280v
^5065   TACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACT----------------CAATTAAATGACAGCTCAGAGGAGGAGGATGAAATAGAT
        TACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACT                CAATTAAATGACAGCTCAGAGGAGGAGGATGAAATAGAT
E7      TACATTGCATGAATATATGTTAGATTTGCAACCAGAGACAACTGATCTCTACTGTTATGAGCAATTAAATGACAGCTCAGAGGAGGAGGATGAAATAGAT
        20^       30^       40^       50^       60^       70^       80^       90^       100^      110^

            290v      300v      310v      320v      330v      340v      350v      360v      370v      380v
^5065   GGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACAC
        GGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACAC
E7      GGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTAACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACAC
          ^       130^      140^      150^      160^      170^      180^      190^      200^      210^

            390v      400v      410v      420v      430v      440v      450v      460v      470v      480v
^5065   ACGTAGACATTCGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAATCTACCATGGCGGATCCAGA
        ACGTAGACATTCGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAA
E7      ACGTAGACATTCGTACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAA
          ^       230^      240^      250^      260^      270^      280^      290^

Similarity index = 99.643%, total bases not opposite a gap = 279
Number of gaps = 1,  bases opposite gaps = 18
Length of the overlapping region = 297
Number of unambiguous matching bases = 279
Number of ambiguous matching bases = 0
```

WB anti-E67sur VVTG5021&5065

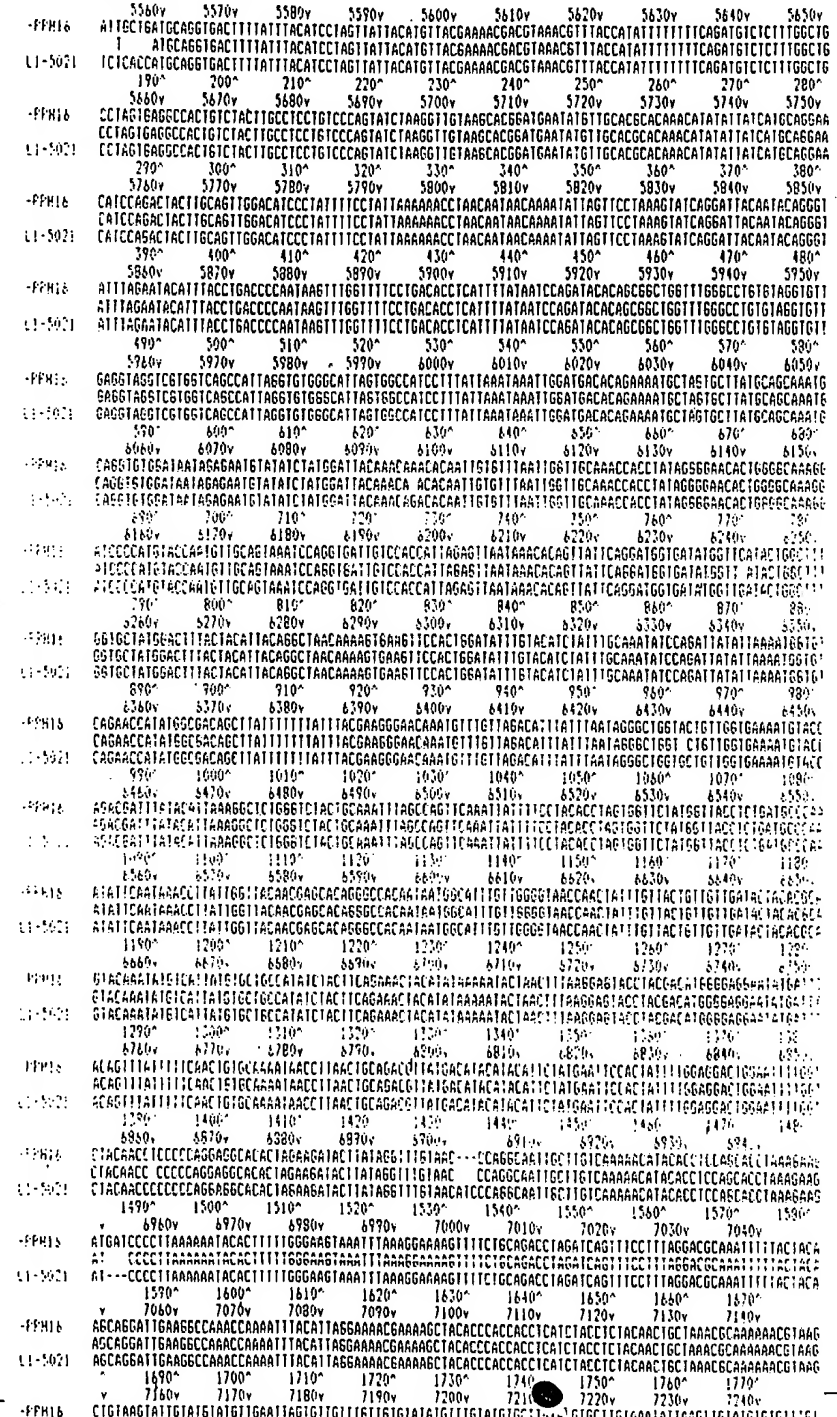


Scientific Council. TRANSGENE SA

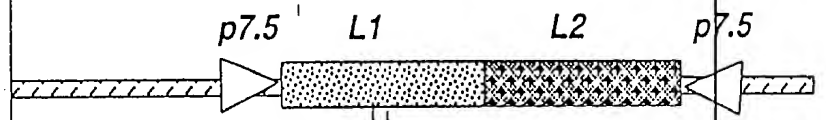
Eval10.95/87/131/132

Figure N°5

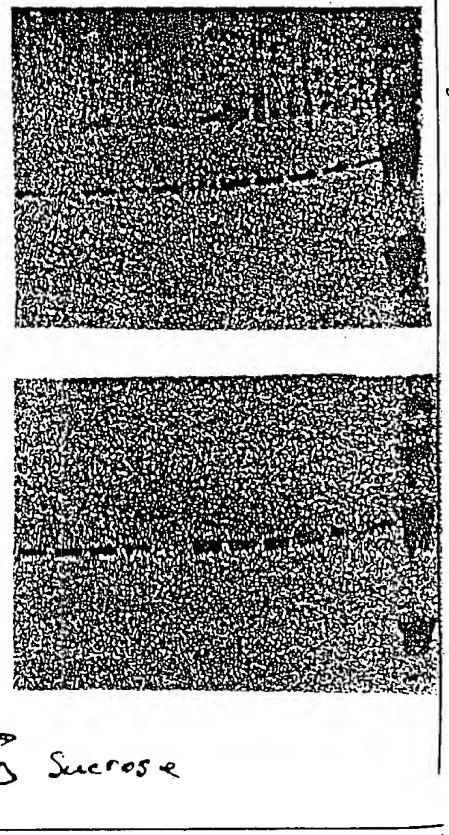
Séquence pTG5021&5065



L1 produite par VVTG5021&5065



WB ICAM-1 sur VVTG5021&5065



Scientific Council. TRANSGENE SA

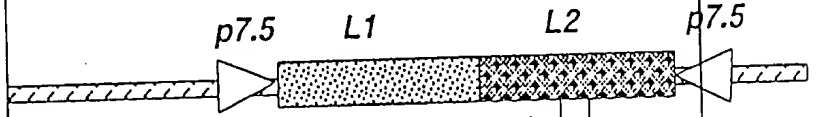
Eval10.95/87/131

Figure N°6

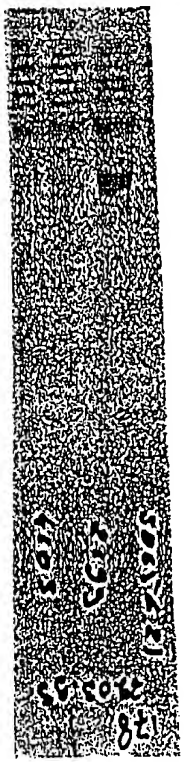
Séquence pTG5021&5065

```
                 v        4230v    4240v    4250v    4260v    4270v    4280v    4290v    4300v    4310v
-PPH16     CTGTTATTA------CTTAACAATGCGACACAAACGTTCTGCAAAAACGCACAAAACGTGCATCGGCTACCCAACTTTATAAAACATGCAAACAGGCAGGT
           C GT A          CTTAAC ATG GACACAAACGTTCTGCAAAACGCACAAAACGTGCATCGGCTACCCAACTTTATAAAACATGCAAACAGGCAGGT
L2-5021    CAGTCAGATCTGGATCTTAACCATGGGACACAAACGTTCTGCAAAACGCACAAAACGTGCATCGGCTACCCAACTTTATAAAACATGCAAACAGGCAGGT
             100^     110^     120^     130^     140^     150^     160^     170^     180^     190^
             4320v    4330v    4340v    4350v    4360v    4370v    4380v    4390v    4400v    4410v
-PPH16     ACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCTGAACAAATATTACAATATGGAAGTATGGGTGTATTTTTTGGTGGGTTAGGAA
           ACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCTGA CAAATATTACAATATGGAAGTATGGGTGTATTTTTTGGTGGGTTAGGAA
L2-5021    ACATGTCCACCTGACATTATACCTAAGGTTGAAGGCAAAACTATTGCTGATCAAATATTACAATATGGAAGTATGGGTGTATTTTTTGGTGGGTTAGGAA
             200^     210^     220^     230^     240^     250^     260^     270^     280^     290^
             4420v    4430v    4440v    4450v    4460v    4470v    4480v    4490v    4500v    4510v
-PPH16     TTGGAACAGGGTCGGGTACAGGCGGACGCACTGGGTATATTCCATTGGGAACAAGGCCTCCCACAGCTACAGATACACTTGCTCCTGTAAGACCCCCTTT
           TTGGAACAGGGTCGGGTACAGGCGGACGCACTGGGTATATTCCATTGGGAACAAGGCCTCCCACAGCTACAGATACACTTGCTCCTGTAAGACCCCCTTT
L2-5021    TTGGAACAGGGTCGGGTACAGGCGGACGCACTGGGTATATTCCATTGGGAACAAGGCCTCCCACAGCTACAGATACACTTGCTCCTGTAAGACCCCCTTT
             300^     310^     320^     330^     340^     350^     360^     370^     380^     390^
             4520v    4530v    4540v    4550v    4560v    4570v    4580v    4590v    4600v    4610v
-PPH16     AACAGTAGATCCTGTGGGCCCTTCTGATCCTTCTATAGTTTCTTTAGTGGAAGAAACTAGTTTTATTGATGCTGGTGCACCAACATCTGTACCTTCCATT
           AACAGTAGATCCTGTGGGCCCTTCTGATCCTTCTATAGTTTCTTTAGTGGAAGAAACTAGTTTTATTGATGCTGGTGCACCAACATCTGTACCTTCCATT
L2-5021    AACAGTAGATCCTGTGGGCCCTTCTGATCCTTCTATAGTTTCTTTAGTGGAAGAAACTAGTTTTATTGATGCTGGTGCACCAACATCTGTACCTTCCATT
             490^     410^     420^     430^     440^     450^     460^     470^     480^     490^
             4620v    4630v    4640v    4650v    4660v    4670v    4680v    4690v    4700v    4710v
-PPH16     CCCCCAGATGTATCAGGATTTAGTATTACTACTTCAACTGATACCACACCTGCTATATTAGATATTAATAATACTGTTACTACTGTTACTACACATAATA
           CCCCCAGATGTATCAGGATTTAGTATTACTACTTCAACTGATACCACACCTGCTATATTAGATATTAATAATACTGTTACTACTGTTACTACACATAATA
L2-5021    CCCCCAGATGTATCAGGATTTAGTATTACTACTTCAACTGATACCACACCTGCTATATTAGATATTAATAATACTGTTACTACTGTTACTACACATAATA
             500^     510^     520^     530^     540^     550^     560^     570^     580^     590^
             4720v    4730v    4740v    4750v    4760v    4770v    4780v    4790v    4800v    4810v
-PPH16     ATCCCACTTTCACTGACCCATCTGTATTGCAGCCTCCAACACCTGCAGAAACTGGAGGGCGATTTTACACTTTCATCATCCACTATTAGTACACATAATTA
           ATCCCACTTTCACTGACCCATCTGTATTGCAGCCTCCAACACCTGCAGAAACTGGAGGGCGATTTTACACTTTCATCATCCACTATTAGTACACATAATTA
L2-5021    ATCCCACTTTCACTGACCCATCTGTATTGCAGCCTCCAACACCTGCAGAAACTGGAGGGCGATTTTACACTTTCATCATCCACTATTAGTACACATAATTA
             600^     610^     620^     630^     640^     650^     660^     670^     680^     690^
             4820v    4830v    4840v    4850v    4860v    4870v    4880v    4890v    4900v    4910v
-PPH16     TGAAGAAATTCCTATGGATACATTTATTGTTAGCACAAACCCTAACACAGTAACTAGTAGCACACCCATACCAGGGTCTCGCCCAGTGGCACGCCTAGGA
           TGAAGAAATTCCTATGGATACATTTATTGTTAGCACAAACCCTAACACAGTAACTAGTAGCACACCCATACCAGGGTCTCGCCCAGTGGCACGCCTAGGA
L2-5021    TGAAGAAATTCCTATGGATACATTTATTGTTAGCACAAACCCTAACACAGTAACTAGTAGCACACCCATACCAGGGTCTCGCCCAGTGGCACGCCTAGGA
             700^     710^     720^     730^     740^     750^     760^     770^     780^     790^
             4920v    4930v    4940v    4950v    4960v    4970v    4980v    4990v    5000v    5010v
-PPH16     TTATATAGTCGCACAACACAACAGGTTAAAGTTGTAGACCCTGCTTTTGTAACCACTCCCACTAAACTTATTACATATGATAATCCTGCATATGAAGGTA
           TTATATAGTCGC CAACACAACA GTTAAAGTTGTAGACCCTGCTTTTGTAACCACTCCCACTAAACTTATTACATATGATAATCCTGCATATGAAGGTA
L2-5021    TTATATAGTCGCGCAACACACAACAAGTTAAAGTTGTAGACCCTGCTTTTGTAACCACTCCCACTAAACTTATTACATATGATAATCCTGCATATGAAGGTA
             800^     810^     820^     830^     840^     850^     860^     870^     880^     890^
             5020v    5030v    5040v    5050v    5060v    5070v    5080v    5090v    5100v    5110v
-PPH16     TAGATGTGGATAATACATTATATTTTTCTAGTAATGATAATAGTATTAATATAGCTCCAGATCCTGACTTTTTGGATATAGTTGCTTTACATAGGCCAGC
           TAGATGTGGATAATACATTATATTTT CTAGTAATGATAATAGTATTAATATAGCTCCAGATCCTGACTTTTTGGATATAGTTGCTTTACATAGGCCAGC
L2-5021    TAGATGTGGATAATACATTATATTTTCCTAGTAATGATAATAGTATTAATATAGCTCCAGATCCTGACTTTTTGGATATAGTTGCTTTACATAGGCCAGC
             900^     910^     920^     930^     940^     950^     960^     970^     980^     990^
             5120v    5130v    5140v    5150v    5160v    5170v    5180v    5190v    5200v    5210v
-PPH16     ATTAACCCTCTAGGCGTACTGGCATTAGGTACAGTAGAATTGGTAATAAACAAACACTACGTACTCGTAGTGGAAAATCTATAGGTGCTAAGGTACATTAT
           ATTAACCCTCTAGGCGTACTGGCATTAGGTACAGTAGAATTGGTAATAAACAAACACTACGTACTCGTAGTGGAAAATCTATAGGTGCTAAGGTACATTAT
L2-5021    ATTAACCCTCTAGGCGTACTGGCATTAGGTACAGTAGAATTGGTAATAAACAAACACTACGTACTCGTAGTGGAAAATCTATAGGTGCTAAGGTACATTAT
             1000^    1010^    1020^    1030^    1040^    1050^    1060^    1070^    1080^    1090^
             5220v    5230v    5240v    5250v    5260v    5270v    5280v    5290v    5300v    5310v
-PPH16     TATTATGATTTAAGTACTATTGATCCTGCAGAAGAAATAGAATTACAAACTATAACACCTTCTACATATACTACCACTTCACATGCAGCCTCACCTACTT
           TATTATGATTT ASTACTATTGATCCTGCAGAAGAAATAGAATTACAAACTATAACACCTTCTACATATACTACCACTTCACATGCAGCCTCACCTACTT
L2-5021    TATTATGATTTGAGTACTATTGATCCTGCAGAAGAAATAGAATTACAAACTATAACACCTTCTACATATACTACCACTTCACATGCAGCCTCACCTACTT
             1100^    1110^    1120^    1130^    1140^    1150^    1160^    1170^    1180^    1190^
             5320v    5330v    5340v    5350v    5360v    5370v    5380v    5390v    5400v    5410v
-PPH16     CTATTAATAATGGATTATATGATATTTATGCAGATGACTTTATTACAGATACTTCTACAACCCCGGTACCATCTGTACCCTCTACATCTTTATCAGGTTA
           CTATTAATAATGG TTATATGATATTTATGCAGATGACTTTATTACAGATACTTCTACAACCCCGGTACCATCTGTACCCTCTACATCTTTATCAGGTTA
L2-5021    CTATTAATAATGGCTTATATGATATTTATGCAGATGACTTTATTACAGATACTTCTACAACCCCGGTACCATCTGTACCCTCTACATCTTTATCAGGTTA
             1200^    1210^    1220^    1230^    1240^    1250^    1260^    1270^    1280^    1290^
             5420v    5430v    5440v    5450v    5460v    5470v    5480v    5490v    5500v    5510v
-PPH16     TATTCCTGCAAATACAACAATTCCTTTTGGTGGTGCATACAATATTCCTTTAGTATCAGGTCCTGATATACCCATTAATATAACTGACCAAGCTCCTTCA
           TATTCCTGCAAATACAACAATTCCTTTTGGTGGTGCATACAATATTCCTTTAGTATCAGGTCCTGATATACCCATTAATATAACTGACCAAGCTCCTTCA
L2-5021    TATTCCTGCAAATACAACAATTCCTTTTGGTGGTGCATACAATATTCCTTTAGTATCAGGTCCTGATATACCCATTAATATAACTGACCAAGCTCCTTCA
             1300^    1310^    1320^    1330^    1340^    1350^    1360^    1370^    1380^    1390^
             5520v    5530v    5540v    5550v    5560v    5570v    5580v    5590v    5600v    5610v
-PPH16     TTAATTCCTATAGTTCCAGGGTCTCCACAATATACAATTATTGCTGATGCAGGTGACTTTTATTTACATCCTAGTTATTACATGTTACGAAAACGACGCTA
           TTAATTCCTATAGTTCCAGGGTCTCCACAATATACAATTATTGCTGATGCAGGTGACTTTTATTTACATCCTAGTTATTACATGTTACGAAAACGACGCTA
L2-5021    TTAATTCCTATAGTTCCAGGGTCTCCACAATATACAATTATTGCTGATGCAGGTGACTTTTATTTACATCCTAGTTATTACATGTTACGAAAACGACGCTA
             1400^    1410^    1420^    1430^    1440^    1450^    1460^    1470^    1480^    1490^
             5620v    5630v    5640v    5650v    5660v    5670v    5680v    5690v    5700v    5710v
-PPH16     AACGTTTACCATATTTTTTTTCAGATGTCTCTTTGGCTGCCTAGTGAGGCCACTGTCTACTTGCCTCCTGTCCCAGTATCTAAGGTTE!AGGCAC56616
           AACGTTTACCATATTTTTTTTCAGATGTCTCTTTGGCTGCCTAGTGAAG G  CT      C  C  C  T  T  G T T
L2-5021    AACGTTTACCATATTTTTTTTCAGATGTCTCTTTGGCTGCCTAGTGAAGGTCTTCTAGAGGTACCGCATCGGATATCGATCTCGATATCG
             1500^    1510^    1520^    1530^    1540^    1550^    1560^    1570^    1580^
```

L2 produite par VVTG5021&5065



WB anti-L2 lapin sur VVTG5021&5065

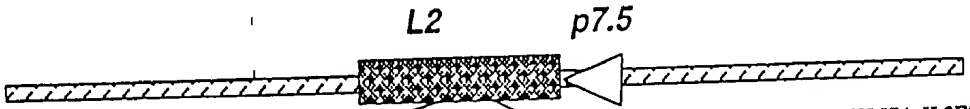


Scientific Council. TRANSGENE SA

Eval10.95/87/131/132

Figure N°7

Il2 produite par VVTG5021&5065



Séquence pTG5021&5065

```
Alignment of:
C:\JMB\CANUTER\PL5065\5065.SEQ     2750 bases from 1 to 2750
                versus
-A14089.SEQ     698 bases from 1 to 698

K-tuple size = 3     range = 20     Gap penalty = 7

                910v     920v     930v     940v     950v     960v     970v     980v     990v     1000v
5065    AATGTACCGCATGCAACTCCTGTCTTGTATCGCCTTAAGTCTCGCACTTGTCACAAACAGCGCTCCTACTTCAAGCTCGACAAAGAAAACACAGCTACAA
                                                                                 G  GACAAAGAAAACACAGCTACAA
-A14089                                                                                 GGGGGGGGGACAAAGAAAACACAGCTACAA
                                                                                        10^      20^      30^
               1010v    1020v    1030v    1040v    1050v    1060v    1070v    1080v    1090v    1100v
5065    CTGGAGCATTTACTTCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGC
        CTGGAGCATTTACTTCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGC
-A14089 CTGGAGCATTTACTTCTGGATTTACAGATGATTTTGAATGGAATTAATAATTACAAGAATCCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGC
                40^      50^      60^      70^      80^      90^     100^     110^     120^     130^
               1110v    1120v    1130v    1140v    1150v    1160v    1170v    1180v    1190v    1200v
5065    CCAAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTT
        CCAAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTT
-A14089 CCAAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTT
                140^     150^     160^     170^     180^     190^     200^     210^     220^     230^
               1210v    1220v    1230v    1240v    1250v    1260v    1270v    1280v    1290v    1300v
5065    AAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACC
        AAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACC
-A14089 AAGACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATCTGAAACAACATTCATGTGTGAATATGCTGATGAGACAGCAACC
                240^     250^     260^     270^     280^     290^     300^     310^     320^     330^
               1310v    1320v    1330v    1340v    1350v    1360v    1370v    1380v    1390v
5065    ATTGTAGAATTTCTGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACTTGATAATTAAGTGCTGTCGAGGAAT------TCACTCCTC
        ATTGTAGAATTTCTGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACTTGATAATTAAGTGCT C A  A     TCA CCT
-A14089 ATTGTAGAATTTCTGAACAGATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACTTGATAATTAAGTGCTTCCCACTTAAAACATATCAGGCCTT
                340^     350^     360^     370^     380^     390^     400^     410^     420^     430^
               1410v    1420v    1430v    1440v    1450v    1460v    1470v    1480v    1490v
5065    AGGTGCAGGCTGCCTATCAGAAGGTGGTGGCTGGTGTGCCAATGCCCTGGCTCACAAATACCACTGAGATCTTTTTCCCTCTGCCAAAAATTATGGGGA
          T  A       T A A  T        TG       T GCT C A T  ACT  AT  TT  C T   C A AA TATGG
-A14089 CTATTTATTTAAATATTTAAATTTTATATTTATTGTTGAATGTATGGTTTGCTACCTATTGTAACTATTATTCTTAAATCTTAAAACTATAAATATGGATC
                440^     450^     460^     470^     480^     490^     500^     510^     520^     530^
               1510v    1520v    1530v    1540v    1550v    1560v    1570v    1580v    1590v
5065    CATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTT-TGTGTCTCTCACTCGGAAG
          T ATGA C  TTG  C A      AA      A TTATTT      AATA T  T  A  TT  TGT     A T  A
-A14089 TTTTATGATTCTTTTTGTAAGCCCTAGGGGCTCTAAAATGGTTTCACTTATTTATCCCAAAATATTTATTATTATGTTGAATGTTAAATATAGTATCTAT
                540^     550^     560^     570^     580^     590^     600^     610^     620^     630^
               1610v    1620v    1630v    1640v    1650v    1660v    1670v    1680v    1690v
5065    GACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCCATATGCTGGCTGCCATGAACAAAGGTTGG
        6   TGG  G AAA ATTTAA A AT GA  A        A A    C C
-A14089 GTASATTGGTTAGTAAAACTATTTAATAAATTTGATAAATATAAACAAAAAAAAAAAAACCCCCCCCC
                640^     650^     660^     670^     680^     690^
```

RESULTATS MANIP ELISA IL2E9506 du 25.01.95

	standard R&D	NIBSC= R&Dx1,55	standard Boehringer
SN VVTG 5058 lot brut = préstock	421,0 ng/ml	652,6 ng/ml	612,0 ng/ml
SN VVTG 5058 stock	545,2 ng/ml	845,1 ng/ml	769,9 ng/ml
SN VVTG 5058 lot purifié	357,2 ng/ml	553,7 ng/ml	518,1 ng/ml
SN ETAtm	0,002 ng/ml	0,003 ng/ml	0,006 ng/ml
SN VVTG 65X21	4,5 ng/ml	7,0 ng/ml	7,7 ng/ml
SN VVTG 61X21	0,003 ng/ml	0,005 ng/ml	0,008 ng/ml
SN WT	0,011 ng/ml	0,017ng/ml	0,02 ng/ml

RESULTATS MANIP FONCTIONNALITE IL2E9507

	standard Boehringer
SN VVTG 5058 lot brut = préstock	798,4 ng/ml
SN VVTG 5058 stock	902,8 ng/ml
SN VVTG 5058 lot purifié	807,5 ng/ml
SN ETAtm	0 ng/ml
SN VVTG 65X21	5,7 ng/ml
SN VVTG 61X21	0 ng/ml
SN WT	0 ng/ml

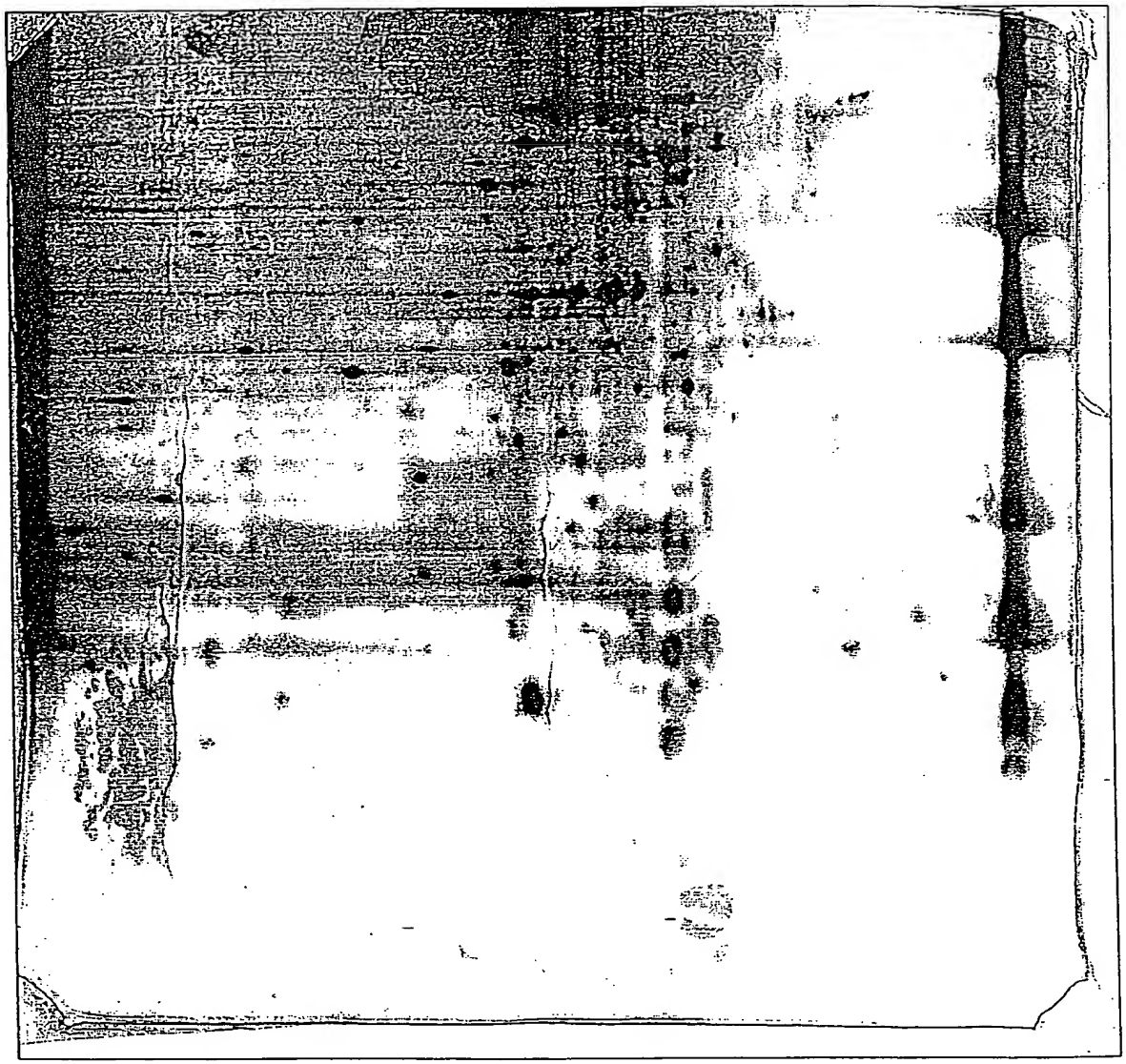
Eval10.95/87/

Scientific Council. TRANSGENE SA.

VVTG5021&5065

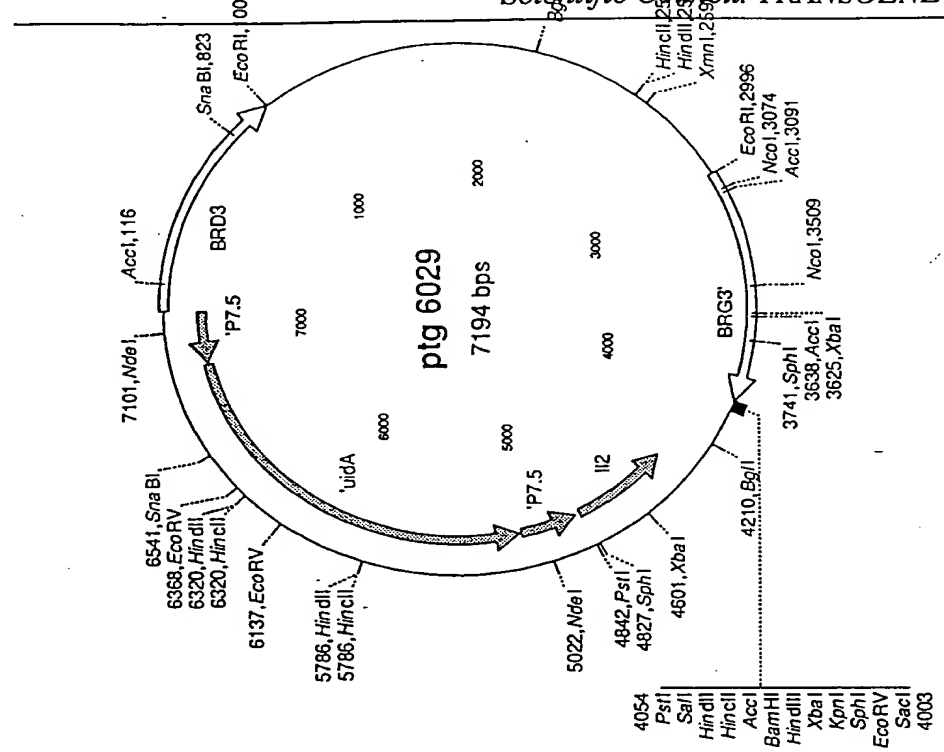
Nitrate d'argent, fixation des petites protéines par la formaldéhyde

Figure 1



Nitrate d'argent, fixation des petits peptides par la formaldehyde

Figure N°9


ptg 6029
7194 bps
BRD3
BRG3'
'uidA
'P7.5
'P7.5
Il2
1000
2000
3000
4000
5000
6000
7000
8000
Bgl 2117
HincII,2552
HindIII,2552
XmnI,2559
EcoRI,2996
NcoI,3074
AccI,3091
NcoI,3509
3741,SphI
3638,AccI
3625,XbaI
4210,BglI
4054
PstI
SalI
HindIII
HincII
AccI
BamHI
HindIII
XbaI
KpnI
SphI
EcoRV
SacI
4003
4601,XbaI
4842,PstI
4827,SphI
5022,NdeI
6137,EcoRV
5786,HindIII
5786,HincII
6368,EcoRV
6320,HindIII
6320,HincII
6541,SnaBI
7101,NdeI
Acc1,116
SnaBI,823
EcoRI,1007

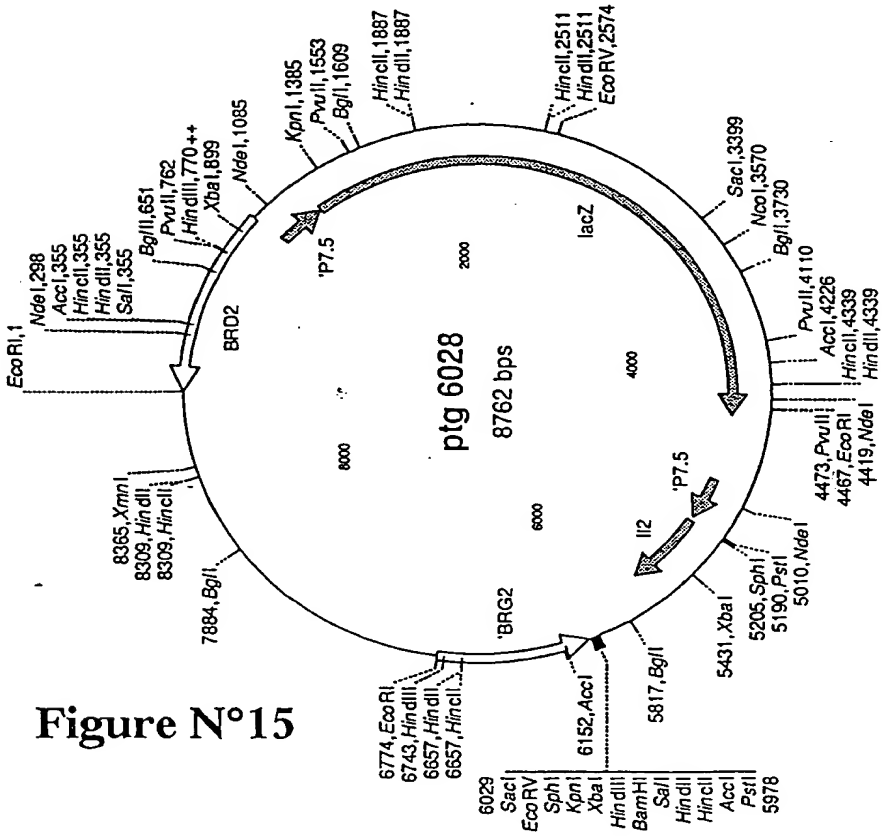

ptg 6028
8762 bps
BRD2
'BRG2
'P7.5
'P7.5
Il2
lacZ
2000
4000
6000
8000
EcoRI,1
NdeI,298
AccI,355
HincII,355
HindII,355
SalI,355
BglII,651
PvuII,762
HindIII,770++
XbaI,899
NdeI,1085
KpnI,1385
PvuII,1553
BglI,1609
HincII,1887
HindII,1887
HincII,2511
HindII,2511
EcoRV,2574
SacI,3399
NcoI,3570
BglI,3730
PvuII,4110
AccI,4226
HincII,4339
HindII,4339
4473,PvuII
4467,EcoRI
4419,NdeI
5010,NdeI
5205,SphI
5190,PstI
5431,XbaI
5817,BglI
6152,AccI
6774,EcoRI
6743,HindIII
6657,HindII
6657,HincII
7884,BglI
8365,XmnI
8309,HindII
8309,HincII
6029
SacI
EcoRV
SphI
KpnI
XbaI
HindIII
BamHI
SalI
HindII
HincII
AccI
PstI
5978

Figure N°15

Nadine Bizouarne, Catherine Pêcheur

Scientific council,

Project 87 : Animal models for vaccinia based vectors for specific cancer immunotherapy.

Infection of mucosal epithilium by papillomaviruses plays a critical role in the development of genital and oral warts and is implicated in the induction of human oropharingeal and cervical cancer. There are actually no vaccine to prevent disease caused by HPV infection. Different levels of problems are encountered in the development of vaccine strategies. First, these viruses are highly species-specific and their *in vitro* culture is, up to now, impossible, making the use of a "simple" animal model out of the question. All the experiments described in this report were done in an "artificial" animal model consisting of mice grafted with tumor cells expressing an HPV target molecule (E7). Second, the HPV uses several strategies to escape its host immune system. In particular i) mucosal lesions caused by HPV induce small quantities of viral particule (the induction of an humoral immune response seems to be only complementary to a cellular one), ii) during the transformation event of the host cell, the viral DNA is integrated to the cellular genome and only the viral early proteins continue to be expressed at low levels, reducing our choice for antigen-specific immunotherapy. As described previously by JM Balloul, a recombinant vaccinia virus expressing E6*E7*L1L2.IL2 (non oncogenic mutated forms of E6 and E7 proteins, VV5021X5065) was produced. In this report we will describe the *in vivo* activities of this vaccinia vector and several approaches used to obtain a second generation vaccinia virus vector.

1- *In vivo* toxicity of VV5021X5065 :

In order to demonstrate the attenuation of the VV5021X5065, we compared the toxicity of this construct to the toxicity of a wild type vaccinia virus. This comparison was made in immunodeficient mice.

1.1 Dose effect : Nude mice were injected intramuscularly with 10^6, 10^7 or 10^8 pfu of VV5021X5065 or VVwt. The number of mice developing vaccinia lesions are indicated in the table 1, and show that even when injected with the non attenuated VVwt, few mice have vaccinia lesions.

1.2 Immunization route effect : Nude mice were injected with 10^7 pfu of vaccinia by different routes of immunization : intracranial , intramuscular, intravenous, or subcutaneous. The table 2 represents the number of mice having vaccinia lesions.

It seems clear that the VV5021X5065 is consistantly attenuated comparing to the VVwt: even when injected by the intracranial route, this recombinant does not lead to vaccinia lesions in this experimental system.

VV WT IV 5/5
IC 4/5
IP 2/5
TH 1/5

VV5021 5065 0/5
0/5
0/5
0/5

2- *In vivo* antiviral effect of Ribavirine on vaccinia virus :

In order to make a clinical trial with a recombinant vaccinia virus, we have to test the ability of an antiviral compound to inhibit the development of vaccinia lesions. As we have previously seen, when mice are injected with VV5021X5065, they do not developed vaccinia lesions (even immunodeficient mice). Therefore, the antiviral efficiency was tested in nude mice injected with VVwt and treated with Ribavirine. The table 3 shows the number of mice with lesions after antiviral treatment, and shows that an *in vivo* efficient anti-vaccinia compound is available.

3- *In vivo* efficacy of VV5021X5065:

The viral construct VV5021X5065 was tested for *in vivo* anti-tumoral activity against cells expressing the E7 molecule, and was compared to VV5021X5061 (E6*E7*L1L2), VVIL2 and VVcontrol (186). This activity was assessed in 2 models : immunoprotection and immunotherapeutic experiments, whose protocols are similar in all experiments described in this report.

3.1 - Immunoprotection experiment : Mice were immunized subcutaneously 3 times with 10^7 pfu of VV5021X5065. Three days after the last immunization, they were challenged with 10^3 E7W1 cells. The percentage of surviving animals in function of time is represented in figure 1 and shows a clear increase for mice immunized with VV5021X5065.

	10^6 pfu	10^7 pfu	10^8 pfu
VVwt	0/5*	1/5	1/5
VV5021X5065	0/5	0/5	0/5

Table 1 : Toxicity of VV5021X5065 compared to VVwt - Dose effect. *number of mice with lesions .

	IV	IP	IM	IC
VVwt	5/5 (2 dead D16)	3/5	1/5	4/5 (4 dead D5)
VV5021X5065	0/5	0/5	0/5	0/5

Table 2 : Toxicity of VV5021X5065 compared to VVwt - Immunization route effect. *number of mice with lesions.

	control		Ribavirine (30mg/kg)		Ribavirine (300mg/kg)	
n° mice	fingers	tail	fingers	tail	fingers	tail
1	0	0	++++	+	0	0
2	0	+	0	+	0	0
3	+++	+++	+++	+	0	0
4	+++	+++	0	0	0	0
5	0	+	+	+	0	0
6	++	+++	+++	++	0	0
7	0	+	++++	++	0	0
8	0	0	+++	++	0	0
9	+++	+++	+	0	0	0
10	+++	+++	0	0	0	0

Table 3 : *In vivo* antiviral effect of Ribavirine on wild type vaccinia virus lesion in nude mice.

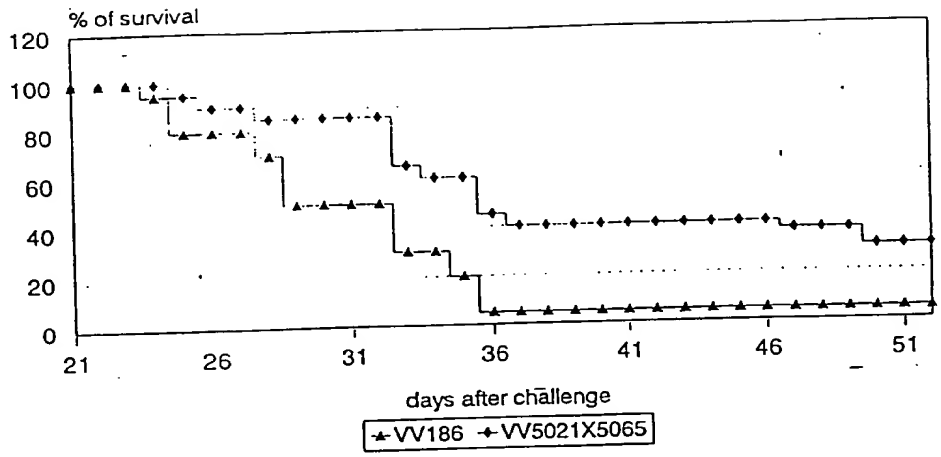
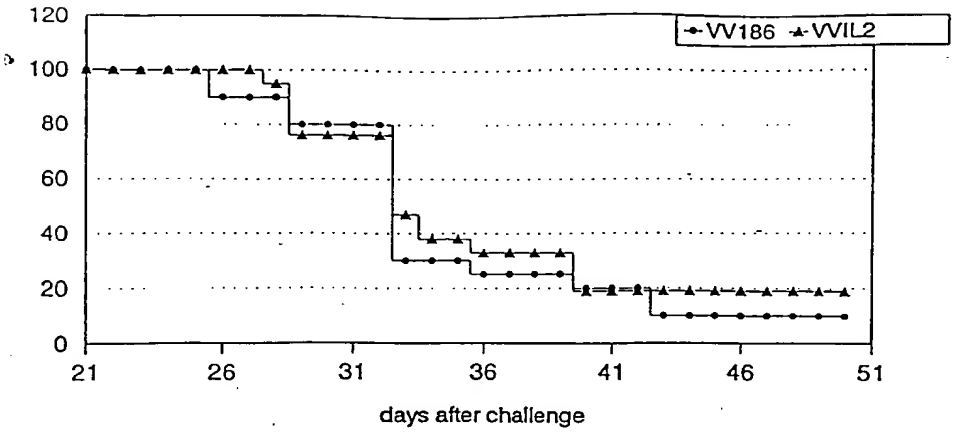
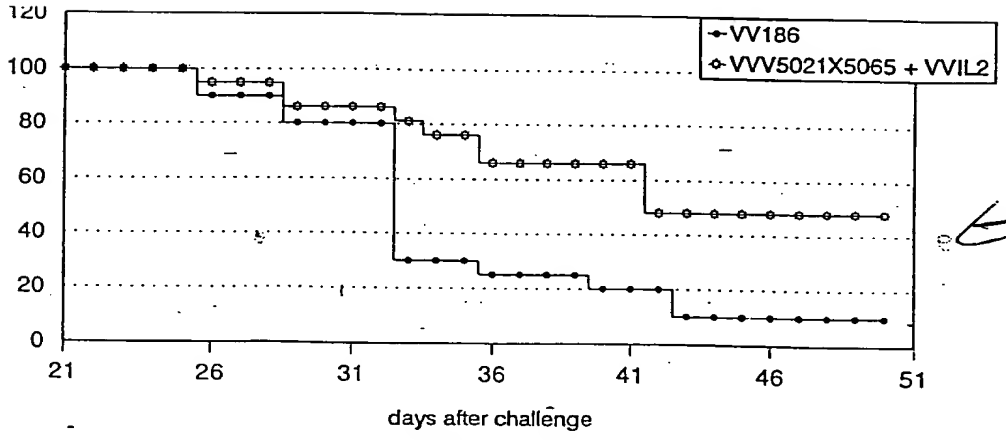


Figure 1 : In vivo efficacy of VV5021X5065. Immunoprotection experiment. 10^7 pfu VV SC, 3X, 10^3 E7W1 SC.





+ combinaison de virus

Figure 7 : Immunotherapy experiment. Effect of IL2 dosage. Mice were injected with 10^3 E7W1 cells and then treated 3 times with 10^7 pfu of VV (SC).